IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

SMITH et al.

Atty. Ref.: **2551-49**

Serial No. **09/662,462**

Group: **1655**

Filed: **September 15, 2000**

Examiner: **Goldberg**

For: **NUCLEIC ACID PROBES AND METHODS FOR DETECTING CLINICALLY IMPORTANT FUNGAL PATHOGENS**

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January 10, 2002

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

AMENDMENT

Responsive to the Official Action dated September 10, 2001, entry and consideration of the following amendments and remarks are requested; the period for response having been extended up to and including January 10, 2002, by submission of the requisite petition and fee, attached.

IN THE SPECIFICATION

Amend the specification as follows.

Page 1, line 1, delete the following paragraph:

"This is a continuation of PCT Application No. PCT/EP00/04714, filed 24 May 2000, the entire contents of which is hereby incorporated by reference in this application. This application claims the benefit of U.S. Provisional Application No.

60/138,621, filed 11 June 1999, the entire content of which is hereby incorporated by reference in this application."

and insert the following therefor:

--This application is a continuation of PCT/EP00/04714, filed 24 May 2000, and claims the benefit of U.S. provisional Application No. 60/138,621, filed 11 June 1999, the entire contents of each of which being incorporated herein by reference.--

Page 37, delete the paragraph spanning lines 7-15 and insert the following therefor:

--PCR amplification of the ITS region was performed in a final volume of 100 µl with 20 µl of DNA extracted from the blood samples (for DNA extracted from 5 ml blood samples, 20 µl of a 1/10 dilution is included in the PCR reaction) added to the PCR reaction containing a final concentration 0.25 mM deoxynucleotidetriphosphates (DU/dNTP's[2:1]), 1x reaction buffer (Promega, USA), 3 mM $MgCl_2$, 1 unit Uracil DNA glycosylase (Longo et al 1990; Roche-Boehringer Mannheim, Germany), 40 pmol each of the forward ITS5 primer (5'-GAAAGTAAAAGTCGTAACAAGG-3') (SEQ ID NO:50) and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (SEQ ID NO:45), 2.5 units of Taq polymerase (Promega, USA), made to a final volume of 100 µl in nuclease free water (Sigma-Aldrich Ltd, UK).--

Insert the attached Sequence Listing for the copy of the same which was filed August 10, 2001.

IN THE CLAIMS

Amend the claims as follows.

Cancel claim 27, without prejudice.

24. (Amended) Method to detect and identify *Candida* species in a sample, comprising at least the following steps:

(i) releasing, isolating and/or concentrating the nucleic acids of fungal pathogens possibly present in the sample,

(ii) if necessary, amplifying the Internal Transcribed Spacer region (ITS) of said nucleic acids with at least one fungal universal primer pair,

(iii) hybridizing the nucleic acids of step (i) or (ii) with at least one of the following species specific oligonucleotide probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2)

TATCAACTTGTCACACCAGA (SEQ ID NO:3)

GTAGGCCTTCTATATGGG (SEQ ID NO:4),

TGCCAGAGATTAAACTCAAC (SEQ ID NO:5),

GGTTATAACTAAACCAAACT (SEQ ID NO:6),

TTTTCCCTATGAACTACTTC (SEQ ID NO:7),

AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),

GAGCTCGGAGAGAGACATC (SEQ ID NO:10),

TAGTGGTATAAGGCGGAGAT (SEQ ID NO:11),

CTAAGGCGGTCTCTGGC (SEQ ID NO:12),

GTTTTGTTCTGGACAAACTT (SEQ ID NO:13),

TTGTCACACCAGATTATTACTT (SEQ ID NO:33),

GGTTTATCAACTTGTCACACCAGA (SEQ ID NO:34),

GGTATCAACTTGTCACACCAGATT (SEQ ID NO:35),

GGTTATAACTAAACCAAACTTTTT (SEQ ID NO:36),

GGGAATATAGCATATAGTCGA (SEQ ID NO:37),

GGTTTTGTTCTGGACAAACTT (SEQ ID NO:38),

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary molecules of said probes,

(iv) detecting the hybridization complexes formed in step (iii), and

(v) identifying the *Candida* species present in said sample, based on the hybridization complex formed.

25. (Amended) Method according to claim 24, wherein the ITS region in step (ii) is limited to the ITS-1 region, and wherein the at least one probe in step (iii) is chosen from the following set of probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2),

TATCAACTTGTCACACCAGA (SEQ ID NO:3),

GTAGGCCTTCTATATGGG (SEQ ID NO:4),

TGCCAGAGATTAAACTCAAC (SEQ ID NO:5),

GGTTATAACTAAACCAAACT (SEQ ID NO:6),

TTTTCCCTATGAACTACTTC (SEQ ID NO:7),

AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),

GAGCTCGGAGAGAGACATC (SEQ ID NO:10),

GTTTTGTTCTGGACAAACTT (SEQ ID NO:13),

TTGTCACACCAGATTATTACTT (SEQ ID NO:33),

GGTTTATCAACTTGTCACACCAGA (SEQ ID NO:34),

GGTATCAACTTGTCACACCAGATT (SEQ ID NO:35),

GGTTATAACTAAACCAAACTTTTT (SEQ ID NO:36),

GGGAATATAGCATATAGTCGA (SEQ ID NO:37),

GGTTTTGTTCTGGACAAACTT (SEQ ID NO:38),

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary nucleic acids of said probes.

28. (Amended) Method according to claim 24 wherein the *Candida* species is *Candida albicans* and wherein the at least one probe of step (iii) is

chosen from among SEQ ID NOs:2, 3, 33, 34 and 35.

29. (Amended) Method according to claim 24 wherein the *Candida* species is *Candida parapsilosis* and wherein the at least one probe of step (iii) is

chosen from among SEQ ID NOs:4 and 5.

30. (Amended) Method according to claim 24 wherein the *Candida* species is *Candida tropicalis* and wherein the at least one probe of step (iii) is

chosen from among SEQ ID NOs:6 and 36.

31. (Amended) Method according to claim 24 wherein the *Candida* species is *Candida kefyr* and wherein the at least one probe of step (iii) is

chosen from among SEQ ID NOs:7 and 8.

32. (Amended) Method according to claim 24 wherein the *Candida* species is *Candida krusei* and wherein the at least one probe of step (iii) is

chosen from among SEQ ID NOs:9 and 37.

33. (Amended) Method according to claim 24 wherein the *Candida* species is *Candida glabrata* and wherein the probe of step (iii) is

SEQ ID NO:10.

34. (Amended) Method according to claim 24 wherein the *Candida* species is *Candida dubliniensis* and wherein the at least one probe of step (iii) is

chosen from among SEQ ID NOs:11, 12, 13 and 38,.

35. (Amended) Method according to claim 24 wherein the at least one probe of step (iii) is immobilized to a solid support.

36. (Amended) Method according to claim 24 for the simultaneous detection and differentiation of at least two *Candida* species in one single hybridization step, including

(i) releasing, isolating and/or concentrating the nucleic acids of the fungal pathogens possibly present in the sample,

(ii) amplifying the Internal Transcribed Spacer region (ITS) of said nucleic acids with at least one fungal universal primer pair,

(iii) hybridizing the nucleic acids of step (i) or (ii) with at least two of the following species specific oligonucleotide probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2),

TATCAACTTGTCACACCAGA (SEQ ID NO:3),

GTAGGCCTTCTATATGGG (SEQ ID NO:4),

TGCCAGAGATTAAACTCAAC (SEQ ID NO:5),

GGTTATAACTAAACCAAACT (SEQ ID NO:6),

TTTTCCCTATGAACTACTTC (SEQ ID NO:7),

AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),

GAGCTCGGAGAGAGACATC (SEQ ID NO:10),

TAGTGGTATAAGGCGGAGAT (SEQ ID NO:11),

CTAAGGCGGTCTCTGGC (SEQ ID NO:12),

GTTTTGTTCTGGACAAACTT (SEQ ID NO:13),

TTGTCACACCAGATTATTACTT (SEQ ID NO:33),

GGTTTATCAACTTGTCACACCAGA (SEQ ID NO:34),

GGTATCAACTTGTCACACCAGATT (SEQ ID NO:35),

GGTTATAACTAAACCAAACTTTTT (SEQ ID NO:36),

GGGAATATAGCATATAGTCGA (SEQ ID NO:37),

GGTTTTGTTCTGGACAAACTT (SEQ ID NO:38),

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary molecules of said probes,

wherein said probes have been immobilized to a solid support on specific locations,

(iv) detecting the hybridization complexes formed in step (iii),

(v) identifying the species present in the sample by the location of the hybridization signal on the solid support.

37. (Amended) Isolated oligonucleotide molecule consisting of a nucleotide sequence represented by any of SEQ ID NOs:2 to 13 or 33 to 38, or the RNA equivalents of said SEQ IDs wherein T is replaced by U, or the complementary nucleic acid of said SEQ IDs.

38. (Amended) Isolated oligonucleotide molecule according to claim 37, for use as a species specific primer or probe in the detection of one of the following fungal pathogens: *Candida albicans, Candida parapsilosis, Candida tropicalis, Candida kefyr, Candida krusei, Candida glabrata,* and *Candida dubliniensis.*

40. (Amended) Method to detect and identify *Candida* species in a sample, comprising at least the following steps:

(i) releasing, isolating and/or concentrating the nucleic acids of fungal pathogens possibly present in the sample,

(ii) if necessary, amplifying the Internal Transcribed Spacer region (ITS) of said nucleic acids with at least one fungal universal primer pair,

(iii) hybridizing the nucleic acids of step (i) or (ii) with at least one of the following species specific oligonucleotide probes:

TGTCACACCAGATTATTACT (SEQ ID NO:2)

TATCAACTTGTCACACCAGA (SEQ ID NO:3)

GTAGGCCTTCTATATGGG (SEQ ID NO:4),

TGCCAGAGATTAAACTCAAC (SEQ ID NO:5),

GGTTATAACTAAACCAAACT (SEQ ID NO:6),

TTTTCCCTATGAACTACTTC (SEQ ID NO:7),

AGAGCTCGTCTCTCCAGT (SEQ ID NO:8),

GGAATATAGCATATAGTCGA (SEQ ID NO:9),

GAGCTCGGAGAGAGACATC (SEQ ID NO:10),

TAGTGGTATAAGGCGGAGAT (SEQ ID NO:11),

CTAAGGCGGTCTCTGGC (SEQ ID NO:12),

GTTTTGTTCTGGACAAACTT (SEQ ID NO:13),

TTGTCACACCAGATTATTACTT (SEQ ID NO:33),

GGTTTATCAACTTGTCACACCAGA (SEQ ID NO:34),

GGTATCAACTTGTCACACCAGATT (SEQ ID NO:35),

GGTTATAACTAAACCAAACTTTTT (SEQ ID NO:36),

GGGAATATAGCATATAGTCGA (SEQ ID NO:37),

GGTTTTGTTCTGGACAAACTT (SEQ ID NO:38),

or the RNA equivalents of said probes, wherein T is replaced by U, or the complementary molecules of said probes,

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(iv) detecting the hybridization complexes formed in step (iii), and

(v) identifying the *Candida* species present in said sample, based on the hybridization complex formed;

said oligonucleotide probes including a homopolymer tail which is added at the 3' or 5' extremity of the probe.

REMARKS

Reconsideration is requested.

The specification has been amended at page 37 as suggested by the Examiner to include sequence identifiers. The specification has been amended to delete amendments requested on the applicants' cover sheet of September 15, 2000, and insert the same subject matter, in response to the Examiner's comment at page 2 of the Office Action dated September 10, 2001 (Paper No. 15).

The specification has been amended to include the attached Sequence Listing. The attached paper and computer-readable copies of the Sequence Listing are the same. A separate Statement to this effect is attached. No new matter has been added.

The claim objections indicated on page 3, paragraph 7 of Paper No. 15 are obviated by the above. Claim 27 has been canceled, without prejudice and claims 28-34 have been rewritten to be dependent from claim 24. Withdrawal of the Rule 75 objection to claims 28, 30, 32 and 34 is requested.

The Section 112, second paragraph, rejection of claims 38 and 40 is obviated by the above amendments. Withdrawal of the Section 112, second paragraph, rejection is requested.

The following Section 102 rejections are obviated by the above amendment to claim 37:

The Section 102 rejection of claims 37 and 38 over Williams (Journal of Clinical Pathology, Vol. 49, No. 1, pages M23-M28);

The Section 102 rejection of claims 37-38 over Botelho (Yeast, Vol. 10, pages 709-717, 1994);

The Section 102 rejection of claims 37-38 over Lin (Genbank Accession Number U10987, March 1996) or Lin (Journal of Clinical Microbiology, Vol. 33, No. 7, pages 1815-1821, July, 1995);

The Section 102 rejection of claims 37 and 38 over Messner (Genbank Accession Number U09325, May 1994);

The Section 102 rejection of claims 37 and 38 over Williams (Genbank Accession Number L47108, September 1995;

The Section 102 rejection of claims 37 and 38 over Lott (U.S. Patent No. 6,242,178, June 5, 2001); and

The Section 102 rejection of claims 37 and 38 over Lott (Genbank Accession No. U96719, August, 1997).

The cited documents fail to teach each and every aspect of the invention of claims 37 and 38, as amended above. Withdrawal of the Section 102 rejections of claims 37 and 38 is requested.

To the extent not obviated by the above, the following Section 103 rejections are traversed:

The Section 103 rejection of claims 24-28 and 30 over Botelho in view of Hogan (U.S. Patent No. 5,595,874);

The Section 103 rejection of claims 24-28, 30 and 32 over Williams in view of Hogan;

The Section 103 rejection of claims 24-33 over Williams and Lin (Genbank Accession No. U10987, March, 1996) or Lin (Journal of Clinical Microbiology, Vol. 33, No. 7, pages 1815-1821, July, 1995) or Messner or Williams in view of Hogan;

The Section 103 rejection of claims 35-36 over Botelho in view of Hogan and Fujita (Journal of Clinical Microbiology, Vol. 33, No. 4, pages 962-967, April, 1995);

The Section 103 rejection of claims 35-36 over Williams in view of Hogan and Fujita;

The Section 103 rejection of claims 35-36 over Williams and Lin (Genbank Accession No. U10987) or Lin (Journal of Clinical Microbiology) or Messner or Williams (Genbank Accession No. L47108) in view of Hogan and Fujita;

The Section 103 rejection of claims 35-36 over Lott in view of Hogan and Fujita;

The Section 103 rejection of claims 35 and 36 over Botelho in view of Hogan and Jordan (U.S. Patent No. 6,017,699);

The Section 103 rejection of claims 35-36 over Williams in view of Hogan and Jordan;

The Section 103 rejection of claims 35 and 36 over Williams and Lin (Genbank Accession No. U10987) or Lin (Journal of Clinical Microbiology) or Messner or Williams in view of Hogan and Jordan;

The Section 103 rejection of claims 35 and 36 over Lott in view of Hogan and Jordan;

The Section 103 rejection of claim 39 Botelho, Hogan, Fujita and Tomblike (U.S. Patent No. 4,617,102);

The Section 103 rejection of claim 39 over Williams in view of Hogan, Fujita and Tomblike;

The Section 103 rejection of claim 39 over Williams, Lin (Genbank Accession No. U10987) or Lin (Journal of Clinical Microbiology) or Messner or Williams and Hogan, Fujita and Tomblike;

The Section 103 rejection of claim 39 over Lott in view of Hogan, Fujita and Tomblike;

The Section 103 rejection of claim 40 over Botelho, Hogan and Shah (U.S. Patent No. 5,558,989);

The Section 103 rejection of claim 40 over Williams, Hogan and Shah;

The Section 103 rejection of claim 40 over Williams, Lin or Lin or Messner in view of Hogan and Shah; and

The Section 103 rejection of claim 40 over Lott in view of Hogan and Shah.

With respect to all of the cited art, the Examiner is urged to appreciate that the applicants have discovered, by selection, for example, specific sequences, from the myriad of possible sequences and fragments, oligonucleotides of a specific length and a specific sequence, which possess specific properties, such as being functional as probes under the same hybridization conditions, in one single assay. P14

In the patent application as filed, these oligonucleotides (as probes) have been described to be functional and to be used, under the same hybridization conditions (same reagents, concentrations, temperatures, etc.), with many other probes (described in the

patent application) originating from many other fungal species (several Aspergillus species and also Cryptococcus and Pneumocystis species).

In other words, each one of these probes has the intrinsic properties that allow it to be used together with the other probes, described in the patent application, for the detection of Candida species, and also for the detection of other fungal species.

None of the cited prior art discloses the alignment of the ITS sequences originating from different fungal species. All the documents constituting the cited prior art deal with different strains of the same species. None of these documents even suggests the possibility to use probes originating from different species in a single assay.

A surprising and unexpected benefit, and hence the patentability and unobviousness of the presently claimed invention is this ability to detect multiple species in a single assay.

The applicants explained this aspect of the originally claimed invention in their Response of February 26, 2001. Unfortunately, the Examiner has forced the applicants to elect an allegedly single invention. This inventive aspect of the disclosed and claimed invention must not be overlooked. The applicants submit that the restriction requirement cannot alter the inherent properties of each probe and therefore cannot alter the patentability of the presently claimed invention.

The claims are submitted to be patentable over the cited art and withdrawal of the Section 103 rejections are requested.

The following specific comments are offered in response to the Examiner's remarks.

In paragraph 16 of Paper No. 15, the Examiner asserts claims 24-28 and 30 would have been obvious over Botelho in view of Hogan.

The applicants note however that if it were true that the ITS sequences of *C. albicans* are disclosed in Botelho, it remains incontestable that there are thousands of possible oligonucleotides (used as probes) which may be selected from these sequences and the document fails to provide clear guidance for one of ordinary skill in the art to have made and used the presently claimed invention.

Hogan teaches, notably through its different examples, how to obtain probes for the detection of a genus or a group of microorganisms and also for detection of a specific species; only the latter being the possibly relevant part in the framework of the presently claimed invention. Therefore these following examples are possibly relevant: 1, 2, 9, 11, 15, 16, 18 and 21.

Comparing the hybridization conditions used in the different cases, notably for examples 1, 11 and 15 (respectively col. 14, lines 1-6, col. 39, lines 26-32 and col. 46, lines 59-64), it appears to the applicants that they are different. For example, the SDS concentrations are different (respectively 5%, 3% and 1%), that the temperatures are different (respectively 72°C, 64°C for probe 2, and 60°C). Moreover, even for the same species (example 11, col. 39, line 32 where the temperatures are different for the two probes) the hybridization conditions may not be the same. In other words, Hogan does not teach how to select probes or teach selected probes or teach or suggest that it

would have been possible to select probes that have the ability to be used in the same hybridization conditions in a single assay, for detection and identification purposes. The same is acknowledged, literally, at col. 8, lines 55 to 57: "To obtain the desired specificity, these probes can be hybridized and assayed under different conditions, including salt concentration and/or temperature".

Therefore, starting with the knowledge of Botelho and using the teaching of Hogan, the person ordinarily skilled in the art would not have been led to select the probes of the presently claimed invention.

This reasoning is true for all of the documents disclosing the ITS sequences and combined with the teaching of Hogan, for example.

The claims are submitted to be patentable over the cited art and a Notice of Allowance is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

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